



Appendix A Claims

1. A method of detecting the presence of an intracellular analyte in one or more cells by flow cytometry, the method comprising:
 - a) fixing and permeabilizing said cells;
 - b) catalyzing the deposition of tyramide in cells comprising said intracellular analyte;
 - c) contacting said cells with a detectable label that directly or indirectly binds to tyramide, whereby cells comprising said intracellular analyte are specifically labeled; and
 - d) detecting a signal from cells comprising said detectable label using a flow cytometric device, whereby said signal is at least 10-fold greater than a signal obtainable by standard flow cytometry methods.
2. A method of detecting the presence of an intracellular analyte in one or more cells by flow cytometry, the method comprising:
 - a) fixing and permeabilizing said cells;
 - b) catalyzing the deposition of tyramide conjugated to a detectable label in cells comprising said intracellular analyte, whereby cells comprising said intracellular analyte are specifically labeled; and
 - c) detecting a signal from cells comprising said detectable label using a flow cytometric device, whereby said signal is at least 10-fold greater than a signal obtainable by standard flow cytometry methods.
3. A method according to claim 1 or 2, wherein said signal is at least 20-fold greater than a signal obtainable by standard flow cytometry methods.
4. A method according to claim 1 or 2, wherein said signal is at least 50-fold greater than a signal obtainable by standard flow cytometry methods.
5. A method according to claim 1 or 2, wherein said catalyzing step comprises:
 - (i) incubating the fixed and permeabilized cells with a binding partner that specifically binds to said analyte, wherein said binding partner is conjugated to an enzyme capable of catalyzing the deposition of tyramide;
 - (ii) removing unbound binding partner from said cells; and
 - (iii) contacting bound binding partner with tyramide, whereby said enzyme catalyzes the deposition of tyramide in cells comprising said intracellular analyte.

6. A method according to claim 5, wherein said binding partner is incubated with said fixed and permeabilized cells in a medium comprising at least about 50% serum.
7. A method according to claim 6, wherein said serum is fetal bovine serum.
8. A method according to claim 7, wherein said medium comprises at least about 95% fetal bovine serum.
9. A method according to claim 8, wherein said medium further comprises about 0.2% saponin.
10. A method according to claim 1 or 2, wherein said cells are permeabilized in a medium comprising saponin.
11. A method according to claim 1 or 2, wherein said cells are permeabilized in a medium comprising methanol.
12. A method according to claim 5, wherein said bound binding partner is contacted with tyramide in a medium comprising an aprotic solvent.
13. The medium of claim 12, wherein said medium comprises at least about 5% of an aprotic solvent selected from the group consisting of acetone, dimethyl sulfoxide, acetonitrile, and dimethyl formamide.
14. A method according to claim 1 or 2, wherein said detectable label is a fluorochrome.
15. A method according to claim 14, wherein said fluorochrome comprises a fluorescent molecule selected from the group consisting of fluorescein, phycoerythrin, CY5, allophycocyanine, Texas Red, Peridenin chlorophyll, and cyanine.
16. A method according to claim 5, wherein said enzyme is selected from the group consisting of hydrolases, peroxidase, oxidase, esterases, glycosidases and phosphatases.
17. A method according to claim 5, wherein said enzyme is horseradish peroxidase.
18. A method according to claim 1 or 2, wherein said catalyzing step comprises:
 - (i) incubating the fixed and permeabilized cells with a first binding partner that specifically binds to said first binding partner, wherein said second binding partner comprises an enzyme, wherein said second binding partner is conjugated to an enzyme capable of catalyzing the deposition of tyramide;
 - (ii) removing unbound second binding partner from said cells; and
 - (iii) contacting bound second binding partner with tyramide, whereby said enzyme catalyzes the deposition of tyramide in cells comprising said intracellular analyte.
19. A method according to claim 18, wherein said second binding partner is an immunoglobulin-enzyme conjugate.

20. A method according to claim 19, wherein said second binding partner is incubated with said fixed and permeabilized cells in a medium comprising at least about 50% serum.
21. A method according to claim 20, wherein said serum is fetal bovine serum.
22. A method according to claim 12, wherein said medium comprises at least about 95% fetal bovine serum.
23. A method according to claim 19, wherein said immunoglobulin-peroxidase, immunoglobulin-hydrolase, immunoglobulin-oxidase, immunoglobulin-glycosidase and immunoglobulin-phosphatase.
24. A method according to claim 23, wherein said immunoglobulin-enzyme conjugate is immunoglobulin-horseradish peroxidase.
25. A method according to claim 1 or 2, wherein said one or more cells are one or more mammalian cells.
26. A method according to claim 25, wherein said one or more mammalian cells are selected from the group consisting of basal cells, epithelial cells, erythrocytes, platelets, lymphocytes, T-cells, B-cells, natural killer cells, granulocytes, monocytes, mast cells, Jurkat cells, neurocytes, neuroblasts, cytomegalic cells, dendritic cells, macrophages, blastomeres, endothelial cells, HeLa cells, tumor cells, interstitial cells, Kupffer cells, Langerhans' cells, Langerhans cells, littoral cells, tissue cells, adipose cells, CHO cells, KFL9, and K562 cells.
27. A method according to claim 1 or 2, wherein said one or more cells are cultured cells.
28. A method according to claim 1 or 2, wherein said intracellular analyte is selected from the group consisting of intracellular cytokines, antigens, viral antigens, nuclear antigens, cytoplasmic antigens, organellar antigens, enzymes, cytoskeletal molecules, glycolipids, lipids, glycans, chaperones, RNA, DNA, messenger RNA, ribosomal RNA, signal transduction proteins, and structural proteins.
29. A method according to claim 1 or 2, wherein said intracellular analyte is not a natural component of said one or more cells.
30. A method according to claim 1 or 2, wherein said intracellular analyte cannot be detected by standard flow cytometry methods.
31. A method according to claim 1 or 2, wherein said one or more cells are obtained from a patient.
32. A method according to claim 13, wherein said signal is correlated to a diagnosis of a disease in said patient.
33. A kit for performing a method according to claims 1 or 2.